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The chromosomal arrangement of two linked actin genes in the sea urchin S. purpuratus

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ABSTRACT

Four distinct actin genes of the sea urchin *Strongylocentrotus purpuratus* have been isolated from a recombinant Charon 4 phage library of genomic DNA. The four genes differ considerably from each other in many of their restriction sites. Two of the four genes are closely linked; they are present in the same fragment of cloned DNA. This fragment has been extensively mapped, and some parts of the DNA have been sequenced. The two linked genes are oriented in the same direction, separated by 7.5 kb of DNA. One has an intron following the CAG that codes for the glutamine residue at position 121 in the amino acid sequence of actin. This represents the fifth distinct site at which introns have been found in actin genes, suggesting that the primordial actin gene had at least 6 exons and 5 introns. The actin genes form a distinctive family in which most introns have apparently been precisely excised from the genes.

INTRODUCTION

The actin family of proteins has evolved from one form in lower eukaryotes to six or more forms in higher animals (1). The six forms include cytoplasmic type actins common to all cells and actins specific for the different types of muscle tissues. The regulation of actin genes in tissue development is of special interest because of this variety of forms, some tissue specific and some not. The genes of the actin family are interesting examples of evolution by gene duplication and divergence. Actin genes have been cloned from *Dictyostelium discoideum, Drosophila,* yeast, and rats, and in some cases the DNA has been sequenced (2-8).

This paper presents the isolation of four different actin genes from the sea urchin *Strongylocentrotus purpuratus* and the mapping of two of these genes which are closely linked in the genome. In one of these an intron has been located following the CAG which codes for glutamine 121 in the amino acid sequence of actin. Introns have previously been found in three *Drosophila* actin genes (4, Fyrberg, E., and Davidson, N., personal

communication) and in the yeast actin gene (5,8). In each case there is a single intron, but the introns are located in different positions. There are no introns in *Dictyostelium* actin genes (2,3). One possible explanation for this variation is that the primordial actin gene originated from six DNA fragments giving rise to five introns and that precise excision of introns has been a frequent occurrence in the evolution of actin genes.

MATERIALS AND METHODS

Source of Recombinant DNA. A sea urchin recombinant phage library was provided by Dr. E. H. Davidson. It had been constructed by the ligation of partial AluI·HaeIII fragments of *Strongylocentrotus purpuratus* DNA into the bacteriophage Charon 4 vector by the method of Maniatis *et al.* (9). A hybrid plasmid subclone containing most of the *Drosophila melanogaster* actin gene from the λDmA2 recombinant phage (4) was obtained from Dr. N. Davidson. The two Sal I restriction fragments from the DmA2 actin gene subclone which were used as probes are 0.8 kb and 1.1 kb and contain the actin coding region (nucleotides 30 to 830 from the initiating AUG) and the 3' noncoding and flanking regions (nucleotides 830 to 1930) respectively.

A hybrid plasmid, pSA38, containing 1.5 kb of cDNA of an actin mRNA from *S. purpuratus* blastulae (6) was provided by Dr. G. T. Merlino. The following restriction sites mapped by Merlino *et al.* (6) in this actin cDNA plasmid were utilized in our work: Eco RI, 0; Bam HI, 0.38; Pst I, 1.55; Eco RI, 1.95; and Pst I, 3.95 kb from the Eco RI site in the pBR322 vector.

Isolation of recombinant phage containing *S. purpuratus* actin genes. The *S. purpuratus* library was screened by the plaque hybridization method of Benton and Davis (10) using as probe the DmA2 actin gene subclone labeled by nick translation by the method of Maniatis *et al.* (11) in the presence of 1 ng of DNAse I. Hybridization was done at 68°C in 0.75 M NaCl, 0.075 M Na citrate (5x SSC). Each recombinant phage containing actin sequences was amplified in KH802 *E. coli* cells and purified on two successive CsCl block (ρ 1.4, 1.6) gradients. The phage were heated in 0.5% SDS, 5 mM EDTA (pH 8) for 15 min at 60°C and the DNA was isolated by phenol:chloroform: isoamyl alcohol extraction (50:50:1) followed by ethanol precipitation. The purity of the phage DNA was analyzed on 0.6% agarose gels buffered with Tris-borate (pH 8.3).

Construction of plasmid subclones from phage with actin genes. Eco RI digested phage DNA was ligated into Eco RI cut pBR325 (12) using DNA ligase

from New England Biolabs. *E. coli* HB101 was transformed with this mixture of hybrid plasmids by the method of Wensink *et al.* (13). Ampicillin resistant, chloramphenicol sensitive transformants were screened by first sizing their Eco RI cut plasmid fragments on 0.8% agarose gels and then transferring these fragments to nitrocellulose filters (14) and hybridizing with nick translated DNA (11) from the starting phage and from the DmA2 actin gene subclone.

Restriction mapping and DNA blot hybridizations. Restriction enzymes were obtained from New England Biolabs and used according to the supplier's specifications. The double enzyme digestions which required different reaction conditions were carried out by adjusting the reaction conditions for the second endonuclease after a 1 hr incubation at 37°C with the first endonuclease. Restriction fragments were analyzed on agarose gels buffered with Tris-borate buffer (pH 8.3) and sized by comparison with Hinf I cut pBR322 and Hind III cut λ DNA. DNA fragments were transferred to nitrocellulose (14) and the resulting DNA blot was prehybridized with 5x SSC, 0.2% Na lauroyl sarcosine, 1x Denhardt's solution (15) for 10 min at 25°C. Hybridization was for 20 hrs at 68°C in prehybridization solution containing 0.5-2.0 x 10^7 cpm/filter of a ^{32}P-labeled DNA probe (4 x 10^7 cpm/μg). The filters were then cleaned with four successive 15 min washes in 3x SSC, 0.5% Na lauroyl sarcosine at 37°C. Filters were covered with plastic wrap and exposed to Kodak XR-5 film for 2-7 days at -70°C.

Hybrid DNA Melting Profiles. Hybrid DNA was formed between a labeled DNA fragment probe and an unlabeled DNA fragment which had been transferred to a nitrocellulose filter by blotting (14). The labeled probe was formed by nick translation (11) of an intact DNA, restriction digestion, agarose gel electrophoresis, elution of the desired band, and concentration of the DNA on a 1 ml DE-52 column. This probe was hybridized to the unlabeled fragment on the nitrocellulose filter for 20 hrs at 50°C in the 5x SSC hybridization buffer described above. After exposure of the nitrocellulose filter to XR-5 film, the filter strip with the hybridized band was cut out and minced into an eppendorf tube. The bound radioactivity was eluted from the filter strip by successive 10 min incubations with 0.5 ml 1x SSC, 0.2% Na lauroyl sarcosine at increasing temperatures up to 100°C. The filters utilized in our experiments contained at least 2500 cpm as measured by Cerenkov radiation.

Sequencing of DNA. The method of Maxam and Gilbert (16) as modified by Smith and Calvo (17) was used for sequencing DNA. Restriction fragments

with 3' underlapping ends were labeled using reverse transcriptase and cleaved to give two unequal fragments. The single labeled fragments were isolated on polyacrylamide gels and sequenced.

RESULTS

Isolation of six recombinant phage with sea urchin actin genes. The *S. purpuratus* library was screened a number of times using a cloned *Drosophila* actin gene DNA as a probe and moderately stringent hybridization conditions (68°C in 5x SSC). A total of 6 recombinant phage were found containing sea urchin actin genes, and these were named λSpA1, λSpA2, etc., in the order of isolation. The DNA from each phage was digested with Hae III and subjected to blot hybridization using the labeled *Drosophila* actin gene DNA as a probe (Fig. 1). From the pattern of labeled phage fragments hybridizing with the actin coding sequences it was apparent that only 3 different types of actin clones had been found. These results were confirmed by examining the ethidium bromide stained patterns of these phage

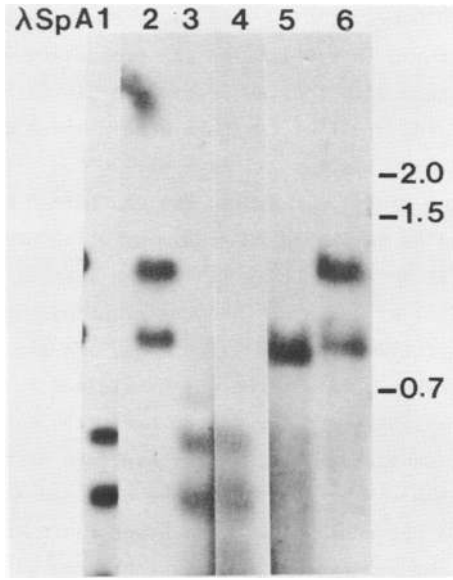

Figure 1. Restriction fragments from 6 recombinant phage containing *S. purpuratus* actin genes bind labeled *Drosophila* actin gene DNA. Phage DNAs from the screening of the *S. purpuratus* library were digested with Hae III, separated on an agarose gel, and subjected to blot hybridization at 68°C in 5x SSC using as probe DNA from the DmA2 subclone (*Drosophila* actin gene) labeled by nick translation. The size of markers in kb are given on the right.

DNAs generated by Eco RI, Hind III, Bam HI, or Sal I cleavage. λSpA1, 3, and 4 contain similar labeled fragments and 2 and 6 contain similar labeled fragments. λSpA2 proved to have two actin genes in a 14 kb DNA fragment, whereas λSpA1 and 5 each contains only 1 actin gene (unpublished). Restriction digest patterns of these four genes showed that they all differ considerably from each other, so it is tentatively concluded that these four genes code for four distinct actins. Complete sequencing of the DNA of each of the four genes will show whether this is indeed the case.

The two linked sea urchin actin genes. The two actin genes linked in λSpA2 were selected for detailed analysis. Since the relation of these two genes and their arrangement in the genome can help us understand the mode of evolution of the actin gene family, the fragment containing the linked genes has been extensively mapped and DNA sequencing has been initiated.

The DNA fragment with the two linked actin genes in λSpA2 is composed of five Eco RI fragments, 1.4, 1.9, 2.0, 3.4, and 5.7 kb in length. These fragments were subcloned into the Eco RI site of pBR325 (12) and the restriction sites in each determined. Two Bam HI sites were located in the 1.4 kb, two Hind III sites in the 3.4 kb, and three Hind III sites in the 1.9 kb Eco RI fragments. Two Sal I, two Hind III, and one Bam HI sites were located in the 5.7 kb Eco RI fragment. The placement of the five Eco RI fragments to form a map of λSpA2 (Fig. 2) was determined by blot hybridization of single and double restriction enzyme digests of the λSpA2 using as labeled probes the individual Eco RI fragments.

To locate the actin coding regions, λSpA2 DNA was digested with Eco RI and a blot hybridization assay was done using labeled 0.8 kb Sal I fragment

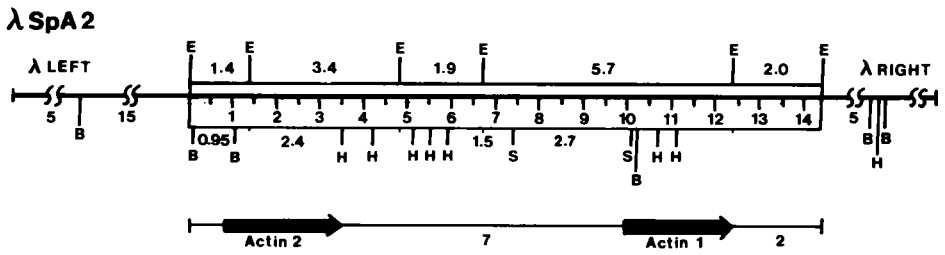

Figure 2. Map of the two linked sea urchin actin genes in the 14 kb insert in a Charon 4 vector. The approximate length and the orientation of transcription of the genes is indicated by heavy arrows. The restriction endonuclease sites are: E, Eco RI; H, Hind III; B, Bam HI; S, Sal I.

of the DmA2 subclone as probe. This probe represents the coding region of a *Drosophila* actin gene (4). Three of the five segments of the 14 kb insert, the 1.4, 3.4 and 5.7 kb Eco RI fragments, hybridize to the *Drosophila* actin coding region probe. When this probe was hybridized to a variety of restriction fragments (Fig. 3), two distinct actin coding regions were localized as shown on the map, Fig. 2. The two genes were numbered 1 and 2. Actin gene 1 coding region is on the right, entirely within the 5.7 kb Eco RI fragment. It is present in all three pieces generated by cleaving this fragment with Hind III. The lack of hybridization to the 2.7 kb Sal I fragment indicates that the coding region does not extend to the left of the Sal I site situated at 10.1 kb. Actin gene 2 lies to the left of actin gene 1. Its coding region does not extend to the right past the Hind III site at 3.5, but does extend leftward slightly past the Bam HI site at 1 kb.

Direction of transcription for actin genes 1 and 2. To determine the orientation of the two actin genes, the probe employed was the cloned cDNA for a cytoplasmic actin mRNA of *S. purpuratus* present in pSA38 (6). The orientation of the cloned cDNA was shown by the fact that the *Drosophila* actin gene coding region hybridizes only to the 1.15 Bam HI•Pst I piece between 0.4 and 1.55 kb from the Eco RI site and that labeled poly d(T) hybridizes to the fragment following the Eco RI site at 1.95 kb. The location of the 3' end of the mRNA just beyond 1.95 was confirmed by DNA sequencing. The 0.4 Pst I•Eco RI piece from 1.55 to 1.95 therefore represents the 3' noncoding region of the mRNA.

To simplify the analysis of the orientation of the actin genes, subclones containing the Eco RI fragments from the 14 kb DNA were employed. One such clone, aSpA2-4, was found to contain the 3.4 and 5.7 kb Eco RI fragments which had been artifactually recombined in the subcloning process. Blot hybridization assay of this subclone with a labeled probe for the 3' noncoding region of actin mRNA shows that actin gene 1 contains a 1.2 kb Eco RI•Hind III fragment which binds the 3' noncoding region of the actin mRNA from *S. purpuratus* blastulae (Fig. 4, channel b). This 3' noncoding region is located to the right of the Hind III site at 11.1 kb. Thus the direction of transcription of actin gene 1 is from left to right. There is not enough homology between actin gene 2 and the 3' noncoding region cDNA probe for binding (Fig. 4).

The orientation of actin gene 2 was determined when it was found that the 5' sequences of actin gene 1 hybridize to the 0.95 kb Bam HI fragment at the extreme left end of the 14 kb DNA (Fig. 5). Thus, we established that

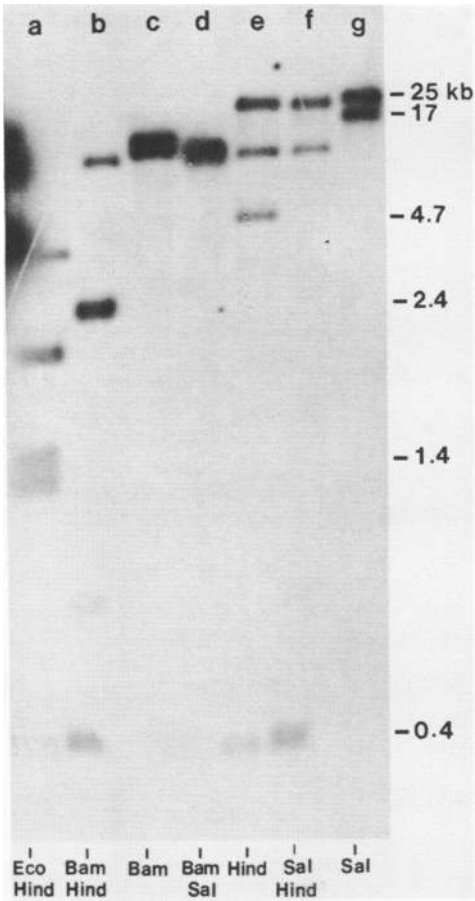

Figure 3

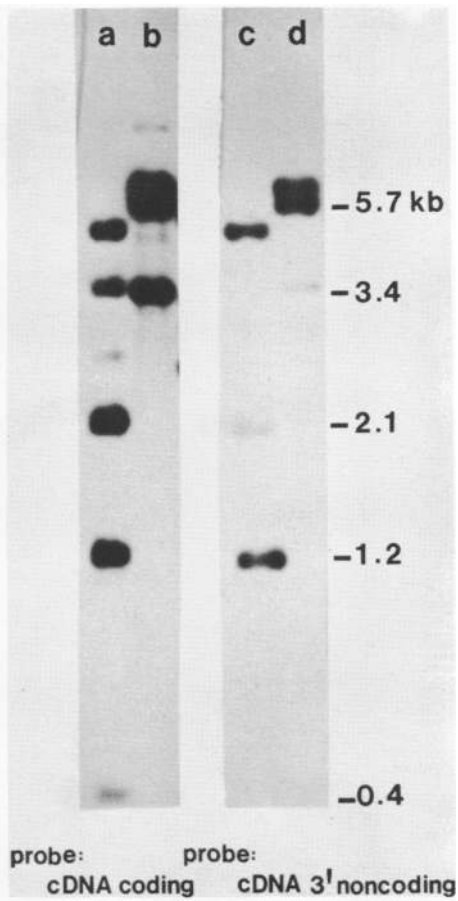

Figure 4

Figure 3. Location of actin coding regions in 14 kb of sea urchin DNA. Blot hybridization assay of λSpA2 with a labeled actin cDNA probe (1.55 kb Eco RI•Pst I fragment of pSA38). The endonucleases utilized were: a) Eco RI•Hind III; b) Bam HI•Hind III; c) Bam HI; d) Bam HI•Sal I; e) Hind III; f) Hind III•Sal I; g) Sal I. Molecular weights of fragments are given at the right.

Figure 4. Location of actin coding and noncoding regions in the 14 kb DNA. Blot hybridization assay of a subclone containing the 3.4 and 5.7 kb Eco RI fragments of λSpA2 (aSpA2-4) with the following labeled fragments of actin cDNA: a and b, 1.55 kb fragment of pSA38 (coding region); c and d, 0.4 kb Eco RI•Pst I fragment of pSA38 (noncoding region). Endonucleases used were: a and c, Eco RI•Hind III; b and d, Eco RI.

both actin genes are transcribed from left to right. In a reciprocal test, a blot hybridization of actin gene 1 was assayed with a probe from the 5' end of actin gene 2 (1.4 kb Eco RI piece) (Fig. 6). This probe binds strongly

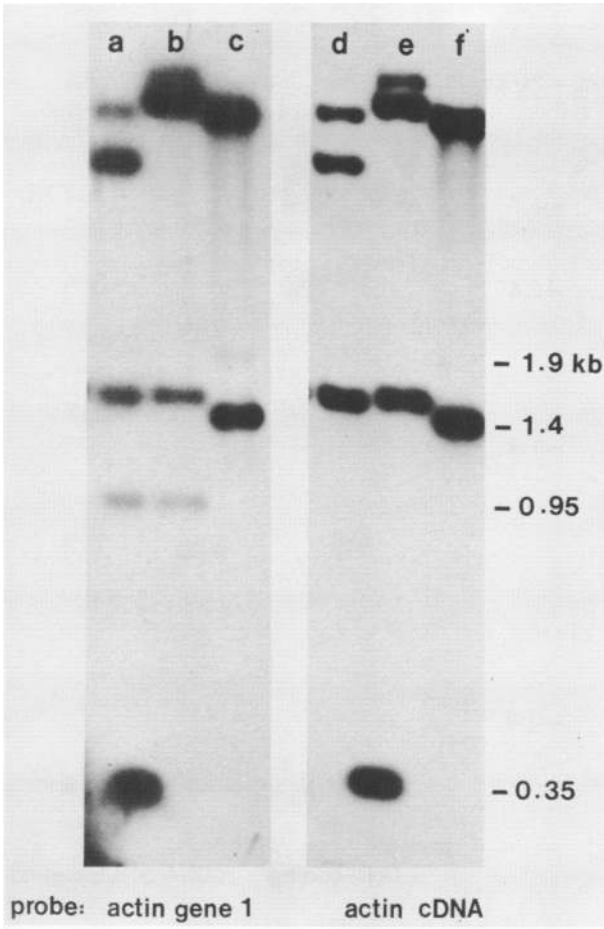

Figure 5

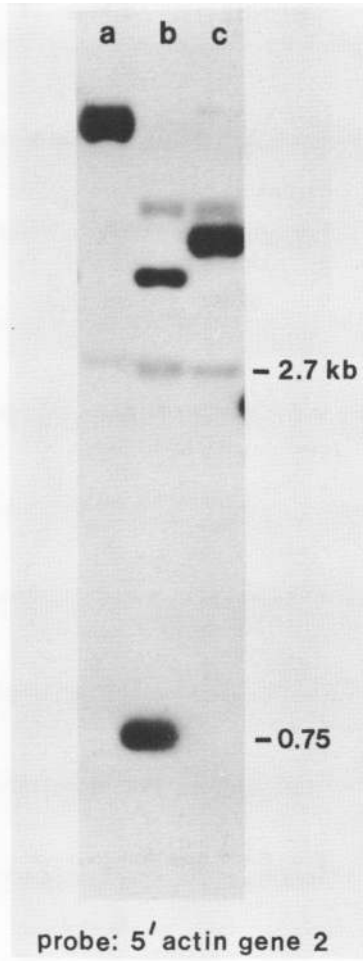

Figure 6

Figure 5. Direction of transcription of actin gene 2. A blot hybridization of aSpA2-8, a subclone containing the 1.4 and 1.9 kb Eco RI pieces of λSpA2, cut with: a and d, Eco RI and Bam HI; b and e, Bam HI; c and f, Eco RI. The two identical blots were hybridized with nick translated labeled probes: a-c, 5.7 kb Eco RI fragment of λSpA2; d-f, 1.55 kb Eco RI•Pst I fragment of pSA38. The 1.5 kb Bam HI and larger fragments contain pBR325 sequences which bind contaminating vector DNA in the probe.

Figure 6. Locating the 5' end of actin gene 1. A blot hybridization of aSpA2-4-2, a subclone containing the 5.7 kb Eco RI fragment of λSpA2, cut with: a, Hind III; b, Sal I•Hind III; c, Sal I. The blot was hybridized with nick translated 1.4 kb Eco RI fragment of λSpA2 at 65°C in 5x SSC.

to sequences between 10.1 and 10.7 kb and weakly to the 2.7 kb Sal I piece to the left of 10.1 kb. These cross hybridizations helped to locate the 5' ends of the 2 actin genes shown on the map. DNA sequencing has confirmed this mapping.

Melting profiles of hybrids between actin genes and actin cDNA. To determine the degree of homology between the structural coding region of the cytoplasmic actin cDNA and actin genes 1 and 2, melting profiles of hybrids were obtained. Fragments of λSpA2 containing either gene 1 or 2 were electrophoretically separated, transferred to nitrocellulose (14) and hybridized to the labeled actin structural coding sequence (1.15 kb Pst I• Bam HI fragment of pSA38). Dissociation of the DNA hybrids with increasing temperature was measured by the release of radioactivity from the nitrocellulose filter piece containing each hybrid. The hybrid with gene 1 dissociates with a T_m of 86°C (Fig. 7), while the hybrid with actin gene 2 dissociates with a T_m of 83°C (not shown). The melting temperature of 86° indicates a very high degree of homology between the coding region of gene 1 and the cytoplasmic actin mRNA. The melting transition is broad because

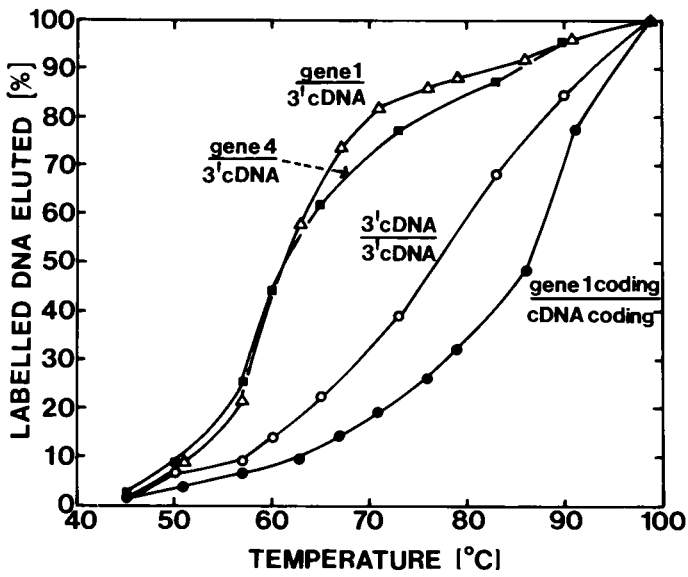

Figure 7. Melting profiles of hybrids between actin cDNA and genes 1 and 4. Labeled 1.15 kb Bam HI•Pst I fragment of pSA38 (actin coding region of cDNA) is eluted from its hybrid with 5.7 kb (●) fragment of λSpA2 (actin gene 1). Labeled 0.4 kb Eco RI•Pst I fragment of pSA38 (3' noncoding region) eluted from its hybrid with itself (○), with 5.7 kb Eco RI fragment of λSpA2 (actin gene 1) (△), or with λSpA5 (actin gene 4) (■).

the probe was slightly fragmented during labeling by nick translation.

A similar test determined the homology between the 3' noncoding region of the cDNA and the four different genes. The hybrid formed between the labeled probe, the 3' noncoding region of the cDNA clone, and actin gene 1 dissociates with a T_m of 62°C (Fig. 7), 15° lower than the T_m of the self-hybrid of the 3' noncoding cDNA. This degree of homology (85%) indicates that actin gene 1 is not the cognate gene of the mRNA represented by pSA38, but is closely related to it. Actin gene 2 does not hybridize with the cDNA 3' noncoding region. In an attempt to find the cognate gene of the cloned actin cDNA, the other two isolated actin genes were tested. Actin gene 4 (from λSpA5) displayed a melting curve similar to that of actin gene 1 (Fig. 7). Actin gene 3 (from λSpA1) did not hybridize with the probe. We conclude that actin genes 1 and 4 are related to the gene for the cloned actin cDNA, and actin genes 2 and 3 are not related to it.

A midgene intron in actin gene 2 of *S. purpuratus*. Further mapping of actin gene 2 indicated the presence of one or more introns, since the coding region is longer than the 1.1 kb required for 374 amino acids. DNA sequencing revealed the presence of an intron following Gln 121. The sequence from Ile 71 through the exon-intron boundary into this intron is shown in Fig. 8. On the restriction map (Fig. 2) the upstream boundary of this intron lies just to the left of the Eco RI site at 1.4 kb. A more detailed restriction map of actin gene 2 indicates that this intron is about 200 to 300 nucleotides long.

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                                    80                                  90
ATC GAG CAC GGT ATC GTC ACC AAC TGG GAC GAT ATG GAG AAG ATC TGG CAT CAC ACC TTC
Ile Glu His Gly Ile Val Thr Asn Trp Asp Asp Met Glu Lys Ile Trp His His Thr Phe
Ile Glu His Gly Ile Ile Thr Asn Trp Asp Asp Met Glu Lys Ile Trp His His Thr Phe

                                   100                                 110
TAC AAC GAA CTC CGT GTT GCC CCA GAG GAG CAC CCC GTC CTC CTT ACC GAG GCT CCC CTC
Tyr Asn Glu Leu Arg Val Ala Pro Glu Glu His Pro Val Leu Leu Thr Glu Ala Pro Leu
Tyr Asn Glu Leu Arg Val Ala Pro Glu Glu His Pro Val Leu Leu Thr Glu Ala Pro Leu

                                   120
                                       CAG GTA AGT
AAC CCC AAG GCC AAC AGG GAA AAG ATG ACA CAG GTA AGA ACA AGC AAA ATG TCC ATT
Asn Pro Lys Ala Asn Arg Glu Lys Met Thr Gln val arg thr ser lys met ser ile
Asn Pro Lys Ala Asn Arg Glu Lys Met Thr Gln Ile Met Phe Glu Thr Phe Asn Val
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Figure 8. DNA sequence of the midgene intron of *S. purpuratus* actin gene 2. The amino acids coded by the sequence are given on line 2. On line 3 are the amino acids in the rabbit muscle actin (18,19). Above Gln 121 is the consensus sequence of the exon-intron boundary (20).

The only published sea urchin gene sequences are those of the histones, and they do not show introns (21,22). Here we show an intron in a sea urchin actin gene with an exon-intron boundary strikingly similar to that in other organisms.

Poly d(CT)•(GA) tracts downstream from each of the two linked actin genes. Within the cluster of sea urchin histone genes, there is a 32 bp long poly d(CT)•(GA) stretch adjacent to the 3' end of the H4 gene and a similar 50 bp stretch at the 3' end of the H2A gene (21,22). To determine whether similar sequences exist near the linked actin genes, ^{32}P labeled poly d(CT) was used as a hybridization probe. Poly d(CT) hybridizes strongly with the 2.0 kb and 1.9 kb fragments of the 14 kb DNA, that is, downstream from each of the 2 linked actin genes. Poly d(CT)•(GA) tracts lie on either side of the Hind III site at 5.9 kb on the map. This means that there are two distinct stretches of poly d(CT)•(GA) downstream from actin gene 2. These tracts are thus adjacent to the 3' end of both the histone and actin genes in sea urchins. However, in the case of the *Drosophila* histone cluster, the poly d(CT)•(GA) tract is adjacent to the 5' end of two of the genes (23). If these tracts are not involved in regulating gene expression, they may contribute to genetic recombination, to the higher structure of chromatin, to the origin of DNA replication, or to the rectification of repeated genes (24).

DISCUSSION

We have isolated four actin genes from the *S. purpuratus* genomic library. Restriction maps of these four show that they are all distinct genes. Two of them (actin genes 1 and 4) hybridize with the 3' noncoding region of the cloned cDNA of a cytoplasmic actin mRNA (6). Melting curves show that their 3' noncoding regions are about 85% homologous with the same region of the cloned cDNA. These genes are related to the cDNA clone but are not cognate to it. The 3' noncoding regions of two other genes have diverged to such an extent that they do not hybridize to the cloned cDNA. We conclude that the *S. purpuratus* genome contains at least five different actin genes, the four described here and the gene cognate to the cloned cDNA (6).

An attempt was made to determine the number of actin genes from a genomic blot, but this method overestimates the number of genes if there are sites in any of the genes for the restriction enzymes used in the analysis. Cleveland *et al.* (25) found 10 bands in a genomic blot of sea urchin DNA hybridized with an actin probe. When the genomic blots of Cleveland *et al.*

(25) are reexamined in the light of the actin gene restriction maps obtained here, it is clear that a total of only five or six actin genes exist in the *S. purpuratus* genome. Thus, the actin genes 1 to 4 described here, together with the cognate gene of the cloned cDNA (6), constitute the majority of the actin genes in the *S. purpuratus* genome.

It appears from our comparison of the 3' noncoding regions of actin genes 1 and 2 that cytoplasmic actin gene 1 is linked to a nonhomologous actin gene. More detailed restriction mapping of the two linked actin genes reveals a direct correlation of many restriction sites in the central coding regions of actin gene 1 and 2. This suggests that these two linked genes may have been formed by gene duplication and that subsequently they diverged extensively in their 3' noncoding regions but only slightly in their coding sequences.

The finding that two actin genes are linked in the *S. purpuratus* genome suggests the possibility that all the actin genes may have remained linked following the initial gene duplication. Clustering of the genes of a single family appears to be common (26). In *Dictyostelium*, some actin genes are linked in DNA fragments (3). The wide dispersal of the six actin genes in *Drosophila* has been the exceptional case (4).

A striking feature of the actin family of genes is the absence of introns in the case of *Dictyostelium* (2,3) and the presence of introns in various locations in other organisms. In *Drosophila*, two genes have been described with introns located in different places near the start of the coding region (4) and another gene has an intron at amino acid 307 (Fyrberg, E., and Davidson, N., personal communication). In yeast a fourth intron has been found in a slightly different location from the *Drosophila* actin introns (5,8). We have reported here that sea urchin actin gene 2 has an intron following Gln 121 in the amino acid sequence, 363 nucleotides downstream from the coding start. This represents the fifth site of an actin gene intron. Actin gene 2 has recently been found to have a second intron in the 5' noncoding region (unpublished). Actin gene 1 has the intron at Gln 121 and a second intron at amino acid 204 (unpublished). Sequencing of these genes is being completed to see if they have any more introns. Mapping and sequencing of the other actin genes is in progress to see what introns they contain.

On finding the intron in the yeast actin gene, Gallwitz and Sures suggested that this intron may have been lost in the evolution of *Dictyostelium* (5). There have been several reports of other types of genes whose

introns have apparently been precisely deleted during evolution (27-29). In the case of actin, the amino acid sequences have been so highly conserved from the single actin in lower eukaryotes (1,5,8) to the six actins in higher organisms (1) that there can be no doubt that the genes for these are all derived from a primordial actin gene. According to the gene shuffling hypothesis (30), the primordial actin gene should have the mid-gene intron found in sea urchins and all the actin introns found in other organisms (4,5,8, and Fyrberg, E., and Davidson, N., personal communication). If this hypothesis is correct, actin genes have lost many introns during evolution; in the case of *Dictyostelium*, all of the introns have been lost, but in other cases one or more of the original introns remain. The presence of introns in different locations in actin genes could also be explained by the hypothesis that introns were inserted into preformed genes (31,32).

Sequence analysis of actin genes 1 and 2 has recently demonstrated that these genes code for N-terminal amino acids similar to those in the vertebrate cytoplasmic actins (unpublished). The functional identification of these genes and genes 3 and 4 will depend, therefore, upon hybridization of their 3' noncoding regions with RNA preparations from different tissues. After this characterization, the gene-specific 3' noncoding region probes will be used for studying the expression of actin genes in the developing sea urchin embryo.

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